Video Article

Assembly of Gold Nanorods into Chiral Plasmonic Metamolecules Using DNA Origami Templates

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Abstract

The inherent addressability of DNA origami structures makes them ideal templates forthe arrangement of metal nanoparticles into complex plasmonic nanostructures. The high spatial precision of a DNA origami-templated assembly allows controlling the coupling between plasmonic resonances of individual particles and enables tailoring optical properties of the constructed nanostructures. Recently, chiral plasmonic systems attracted a lot of attention due to the strong correlation between the spatial configuration of plasmonic assemblies and their optical responses (e.g., circular dichroism [CD]). In this protocol, we describe the whole workflow for the generation of DNA origami-based chiral assemblies of gold nanorods (AuNRs). The protocol includes a detailed description of the design principles and experimental procedures for the fabrication of DNA origami templates, the synthesis of AuNRs, and the assembly of origami-AuNR structures. In addition, the characterization of structures using transmission electron microscopy (TEM) and CD spectroscopy is included. The described protocol is not limited to chiral configurations and can be adapted for the construction of various plasmonic architectures.

Introduction

DNA nanostructures, DNA origami in particular, have been widely used to arrange molecules and other nanoscale components (e.g., proteins and nanoparticles [NPs]), with nanometer precision into almost arbitrary geometries[1,2,3,4,5]. The ability to arrange metal NPs on DNA origami templates with a high yield and accuracy enables the fabrication of plasmonic structures with novel optical properties[6,7,8,9,10]. DNA origami technique is especially useful for the generation of chiral plasmonic structures, which require genuinely three-dimensional architectures[11,12,13,14,15,16,17,18,19,20].

This protocol describes in detail the entire process of the fabrication of DNA origami-templated chiral assemblies of AuNRs. The software used for the design[21] and structure prediction[22,23] of DNA origami is intuitive and freely available. The origami fabrication and AuNR synthesis use common biochemistry lab equipment (e.g., thermocyclers, gel electrophoresis, hot plates, centrifuges). The structures are characterized using standard TEM and CD spectroscopy.

The fabrication of similar plasmonic nanostructures with top-down methods (e.g., electron beam lithography) would require rather complicated and expensive equipment. In addition, DNA origami templates provide the possibility to incorporate structural reconfigurability in plasmonic assemblies[24,25,26,27,28,29,30,31,32,33], which is extremely challenging for structures fabricated with lithography techniques. Compared to other molecular-based approaches[34,35,36,37], DNA origami-based fabrication provides a high level of spatial precision and programmability.

Protocol

1. Design of the DNA origami

1. Identify the desired relative spatial arrangement of AuNRs and the suitable shape of the DNA origami template (**Figure 1A**). Estimate the structural parameters of the AuNRs and the origami templates. Locate the approximate positions of staples that need further modification (**Figure 1B**).
2. Download and install caDNAno[18] to design a DNA origami template. In caDNAno, route the scaffold strand according to the desired shape of the template and generate the staple strands by clicking **Seq Tool**. Click **Paint Tool** and mark the staple strands that require further modification (**Figure 1C**).
3. Click **Export Tool** to export the DNA staple sequences (**Figure 1C**) to a .csv file.
4. Design double-stranded locks to fix the angle Θ between the two origami bundles. Depending on the relative orientation of the two bundles, the origami construct can adapt left- or right-handed (LH/RH) chiral spatial configuration (**Figure 1B**).
5. Import the staples' .csv file into a spreadsheet application. Add a polyA$_{10}$ sequence at the end of the staples used for AuNR assembly (handles). Modify the staple strands on the designed lock sites with lock sequences.

NOTE: The assemblies in the representative results contain 36 handles protruding at the 3' end of the staple strands, 18 on each DNA origami bundle, equally distributed on two parallel helices every 21 nt. The distance between the first and the last handle position is 168 nt, approximately 57 nm (see the attached caDNAno file).

2. Assembly of the DNA origami templates

1. Prepare a working stock of staple stands (SM), including strands with handles and locks, by mixing equal amounts of concentration-normalized staple oligonucleotides (e.g., 100 µM).
   NOTE: Origami structures usually contain several hundreds of staple strands. Staples are typically purchased from vendors specializing in the chemical synthesis of DNA oligonucleotides in multiwell (e.g., 96-well) plates.
2. For 500 µL of 10 nM origami, mix 50 µL of Tris-EDTA (TE, 10x), 100 µL of $MgCl_2$ (100 mM), 25 µL of NaCl (100 mM), 170 µL of H_2O, 100 µL of SM (0.5 µM), 5 µL of lock strands (5 µM), and a 50 µL scaffold (100 nM).
3. Anneal the mixture in a thermocycler from 80 °C to 20 °C as described in **Table 1**.

3. DNA origami purification

NOTE: This section describes the protocol for agarose gel purification. DNA origami templates can also be purified using alternative approaches[38,39].

1. For 1% gel, dissolve 1 g of agarose in 100 mL of Tris-borate-EDTA (TBE, 0.5x) by heating the mixture in a microwave oven. Add 10,000 µL of 10x DNA stain according to the stain specification. To minimize the exposure to UV light at the extraction step (step 3.6), use a DNA stain that can be visualized under blue excitation.
2. Cool the solution to approximately 40 °C and slowly add 1 mL of $MgCl_2$ (1.3 M) while shaking. Cast gel and incubate for 30 min at room temperature.
3. Set the electrophoresis device and pour cold (4 °C) running buffer (0.5x TBE with 11 mM $MgCl_2$) into the gel box. Place the gel box in an ice water bath.
4. Add loading buffer to the origami samples (6x loading buffer contains 15% polysucrose 400 and 0.25% bromophenol blue in water). Load the samples into the wells with a proper volume according to the comb used (e.g., 50 µL for an 8-well comb of 1.5 mm in thickness).
5. Run the electrophoresis for 2 h at 80 V.
   NOTE: To characterize the origami and separate the open and closed structure, use 2% gel instead of 1% and prolong the running time to 4 h.
6. Image the gel with the gel imager (**Figure 2**). Use a blue light transilluminator to visualize the bands, cut the origami band, smash the gel on a parafilm, and extract the liquid. The recovery yield is approximately 40%.
7. Pipette the liquid into a centrifugal filter unit and spin at 3,000 x *g* for 5 min. Measure the absorption of the origami solution at 260 nm with a UV-visible (UV-VIS) spectrometer. Estimate the concentration of origami using an extinction coefficient of 1.3 x 10^8 $M^{-1}{\cdot}cm^{-1}$.
   NOTE: The typical concentration of origami solution after agarose gel purification is 1-2 nM.
8. Store the purified origami templates at 4 °C for later use.

4. Synthesis of gold nanorods

NOTE: The protocol for AuNR synthesis is adapted from previous literature[40] with minor modifications.

1. Wash all glassware with aqua regia for 5 min, rinse it with water, sonicate it with ultrapure water, and dry it before use.
2. Prepare 0.2 M hexadecyltrimethylammonium bromide (CTAB), 1 mM $HAuCl_4$, 4 mM $AgNO_3$, 64 mM L(+)-ascorbic acid, and 6 mM $NaBH_4$. Use cold water (4 °C) to dissolve $NaBH_4$ and keep it in a fridge at 4 °C. Ascorbic acid solution has to be freshly prepared.
   CAUTION: CTAB is hazardous in case of skin contact (irritant), eye contact (irritant), ingestion, and inhalation. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. $NaBH_4$ is extremely hazardous in case of skin contact (irritant), eye contact (irritant), ingestion, and inhalation. Wear splash goggles, a lab coat, gloves, and a vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent.
3. Prepare Au seeds.
   1. Add 500 µL of CTAB (0.2 M), 250 µL of ultrapure water, and 250 µL of $HAuCl_4$ (1 mM) into a glass vial. Stir at 450 rpm at room temperature for 5 min.
   2. Increase the stirring rate to 1,200 rpm. Add 100 µL of cold $NaBH_4$ solution (6 mM, 4 °C). After 2 min, stop the stirring and incubate the solution in a water bath at 30 °C for 30 min before use.
4. Prepare AuNRs.
   1. Dissolve 0.55 g of CTAB and 0.037 g of 2,6-dihydroxybenzoic acid in 15 mL of warm water (60-65 °C) in a round-bottom flask. Cool down the solution to 30 °C, add 600 µL of $AgNO_3$ (4 mM), and stir at 450 rpm for 2 min. Then, leave the solution undisturbed for 15 min at 30 °C.
   2. Add 15 mL of $HAuCl_4$ (1 mM) to the solution, and stir at 450 rpm for 15 min. Add 120 µL of L(+)-ascorbic acid (64 mM), and then, immediately, stir at 1,200 rpm for 30 s. Add 12 µL of Au seeds, and keep stirring at 1,200 rpm for 30 s.
   3. Incubate the solution in a water bath at 30 °C for 18 h. Do not disturb the solution and use a cap to close the flask.
   4. Transfer the resultant solution to centrifuge tubes, and centrifuge at 9,500 x *g* for 12 min at 20 °C. Discard the supernatant, disperse the pellet in 20 mL of ultrapure water, and perform one more centrifugation step.
   5. Disperse the final pellet in 3 mL of distilled water. Estimate the concentration of AuNRs from a UV-VIS absorption measurement using the extinction coefficient for the longitudinal plasmon resonance. The extinction coefficient can be predicted using AuNR shape parameters[41]. Store the AuNRs at 4 °C for further use.

5. Functionalization of gold nanorods with single-stranded DNA

NOTE: This section describes the protocol for AuNR functionalization with single-stranded DNA (ssDNA), following the so-called low pH route adapted from previous literature[42]. The AuNRs covered with DNA are purified by centrifugation; alternatively, the purification can be performed using agarose gel electrophoresis.

1. Incubate 20 µL of thiol-functionalized polyT DNA strands (1 mM) with 20 µL of freshly prepared tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 14 mM) for 1 h to reduce disulfide bonds.
   NOTE: The thiol groups form bonds with AuNRs, and the polyT sequence hybridizes with the $polyA_{10}$ handle on the origami, in which too many or too few base pairs may lead to a malfunction or an unstable assembly.
   CAUTION: TCEP can cause severe skin burns and eye damage. Wear protective gloves/protective clothing/eye protection/face protection.
2. Mix 150 µL of AuNRs (10 nM) and 40 µL of TCEP-treated thiol-DNA (0.5 mM). Add 1% sodium dodecyl sulfate (SDS) to the AuNR solution to reach a final SDS concentration of 0.05%. Adjust the pH to 2.5-3 with ~1 µL of HCl (1 M).
3. Add 40 µL of TCEP-treated thiol-DNA (0.5 mM) to the AuNR solution. Incubate for 2 h while shaking at 70 rpm.
   NOTE: The AuNR-to-DNA ratio should be in the order of 1:5,000-15,000, depending on the size of the rods. For the AuNRs (70 x 30 nm) prepared following the protocol described in section 4, a 13,000 excess of thiol-DNA is recommended.
4. Add NaCl to reach a final NaCl concertation of 0.5 M and incubate for 4 h at room temperature while shaking at 70 rpm.
   NOTE: A color change at this step may indicate a failed DNA functionalization.
5. Adjust the pH to ~8.5 with TBE buffer (10x) and incubate overnight.
6. Wash the DNA-AuNRs 4x by mixing the samples with 1,000 mL of washing buffer (0.5x TBE with 0.1% SDS), and centrifuge at 7,000 x *g* for 30 min. Remove the supernatant and resuspend the DNA-AuNRs in the remaining liquid (~40 µL). Estimate the concentration of DNA-AuNRs from a UV-VIS absorption measurement as described in step 4.4.5.
   NOTE: The solution might become slightly 'cloudy' at steps 5.3-5.4 due to the CTAB replacement from the surface of the AuNRs by thiol-DNA. The solution should become clear upon warming up to ~35 °C for 5 min.

6. Assembly of gold nanorods on DNA origami templates

1. Add $MgCl_2$ to the solution of purified DNA-AuNRs, to a final concentration of 10 mM. Mix the purified DNA-AuNRs and origami to a 10:1 ratio.
   NOTE: A lower ratio may decrease the product yield[43].
2. Anneal the mixture in a mixer with a temperature control from 40 °C to 20 °C while shaking at 400 rpm, following the procedure in **Table 2**.
   NOTE: For CD characterization, the sample can be measured after this step without further purification.
3. Use 0.7% agarose gel electrophoresis (3.5 h at 80 V) to purify the final origami-AuNR structures.
4. Use a white light transilluminator for imaging. Cut the product band (origami-AuNR dimer) (**Figure 3**), smash the gel on a parafilm, and extract the liquid. Pipette the liquid into a centrifugal filter unit and spin at 3,000 x *g* for 5 min. Resuspend the origami-AuNRs in the solution. The recovery yield from the gel is approximately 50%.
5. Estimate the concentration of the origami-AuNR structures from a UV-VIS absorption measurement as described in step 4.4.5.

7. Transmission electron microscopy imaging

NOTE: This uranyl formate (UFo) staining protocol is adapted from previous literature[44].

1. Mix 200 µL of UFo solution (0.75%) and 1 µL of NaOH (5 M) and vortex immediately for 2-3 min. Centrifuge the stain solution for 3-4 min at 14,000 x *g*. Protect the stain from light exposure (e.g., by wrapping it in aluminum foil).
   CAUTION: UFo is toxic if inhaled or swallowed and can cause eye irritation. In the case of brief exposure or low pollution, use a respiratory filter device. In the case of intensive or longer exposure, use a self-contained respiratory protective device. Wear gloves. The glove material has to be impermeable and resistant to UFo and its solutions. Wear tightly sealed goggles.
2. Glow-discharge carbon/formvar-coated TEM grids for 6 s just before use to increase hydrophilicity and promote the sticking of the structures. Pipette 5 µL sample drops on the TEM grid, incubate for 5-8 min, and remove the drop by gently touching a filter paper with the edge of the grid.
3. Pipette one big (~20 µL) and one small (~10 µL) drop of the stain solution on a parafilm.Put the grid on the small stain solution drop and dry immediately by touching the filter paper with the edge of the grid. Then, put it on the big stain solution drop for 30 s.
4. Remove the liquid on the grid by touching the filter paper with the edge of the grid. Place the grid in the grid holder. Wait for the grid to dry for at least 10 min.
5. Characterize the samples of origami (**Figure 4**), AuNRs (**Figure 5**), and origami-AuNRs (**Figure 6**) by TEM.

8. Circular dichroism measurement

1. Purge the CD spectrometer with N_2 for 20 min.
   NOTE: Most of the CD spectrometers require purging with N_2 before lamp ignition. Check the CD spectrometer manual.
2. Set the bandwidth, scanning range, and acquisition step.
   NOTE: The scanning range depends on the optical properties of AuNRs, which depend on the size of the AuNRs.
3. Measure blank CD with buffer.
4. Measure the CD spectra of origami-AuNR samples (**Figure 7**).
   NOTE: Use quarts or glass cuvettes for CD measurement. Plastic cuvettes are unsuitable for CD spectroscopy. Also, most CD spectrometers allow the simultaneous acquisition of absorption and CD data.

Representative Results

TEM images of DNA origami templates, AuNRs, and final origami-AuNR assemblies are shown in **Figure 4**, **Figure 5**, and **Figure 6A**, respectively. Due to their binding preference to TEM grids, origami-AuNR assemblies are usually seen as parallel origami bundles and rods (**Figure 6A**). Thermal annealing is required for the correct alignment of AuNRs on origami templates (**Figure 6A,B**). The protocol enables high yields of the assembly of AuNRs into chiral metamolecules with strong plasmonic CD responses (**Figure 7**).

Temperature (°C)	Time
80	15 min
79 - 71	1 °C / 1 min
70 - 66	1 °C / 5 min
65 - 60	1 °C / 30 min
59 - 37	1 °C / 60 min
36 - 30	1 °C / 15 min
29 - 20	1 °C / 5 min
20	Hold

Table 1: Temperatures and rates for the thermal annealing of DNA origami templates.

Temperature (°C)	Time (min)
40	130
36	180
32	180
22	Hold

Table 2: Temperatures and holding times for the annealing of AuNRs and DNA origami templates. The cooling rate between the steps is set at 0.1 °C/min. The DNA origami-AuNR samples are annealed while shaking at 400 rpm.

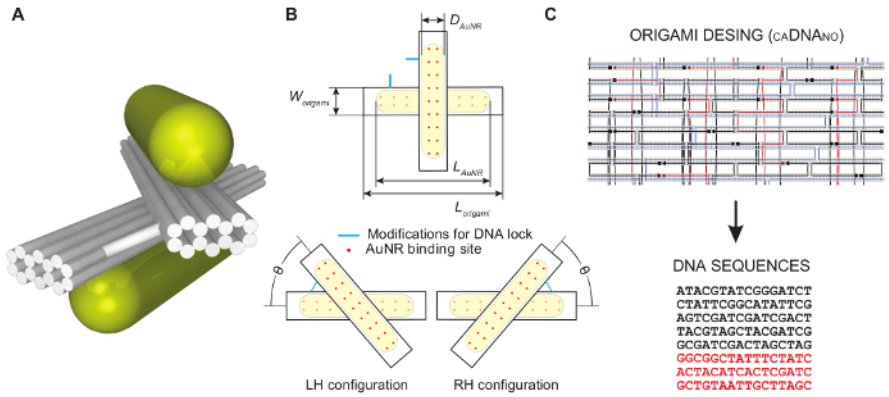

Figure 1: Design of DNA origami-templated chiral metamolecules. (**A**) Identify the desired relative spatial arrangement of gold nanorods (AuNRs) and a suitable shape of the DNA origami template. (**B**) Estimate the structural parameters of the AuNRs (D_{AuNR}, L_{AuNR}) and the origami template ($W_{origami}$, $L_{origami}$,Θ). Locate the approximate positions of the staples that need further modification. (**C**) Design of DNA origami templates using caDNAno. Please click here to view a larger version of this figure.

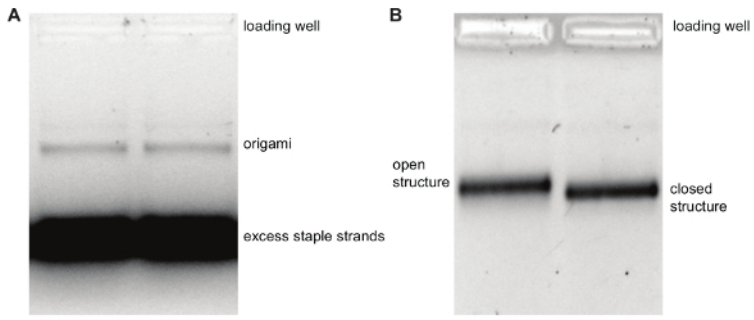

Figure 2: The agarose gel electrophoresis of origami. (**A**) Purification with 1% agarose gel electrophoresis for 2 h at 80 V. (**B**) Characterization with 2% agarose gel electrophoresis for 4 h at 80 V. Please click here to view a larger version of this figure.

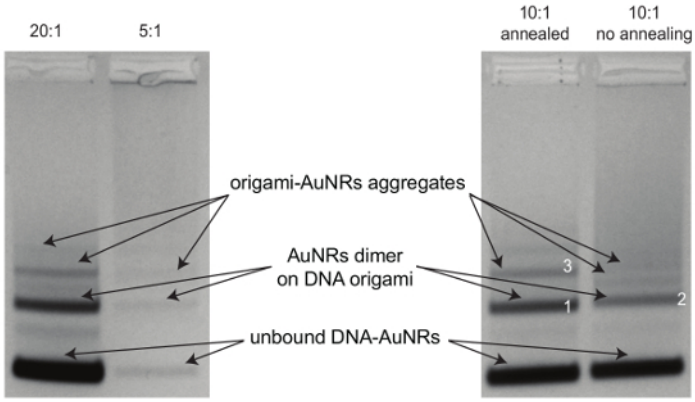

Figure 3: The agarose gel electrophoresis purification of origami-AuNRs. Gel (0.5%) was run for 3.5 h at 80 V for the samples prepared following the assembly procedure with different DNA-AuNR-to-origami ratios (20:1, 5:1) and samples (10:1 DNA-AuNRs-to-origami ratio) with/without annealing procedure. For TEM images of the samples in bands 1, 2, and 3, see **Figure 6**. Please click here to view a larger version of this figure.

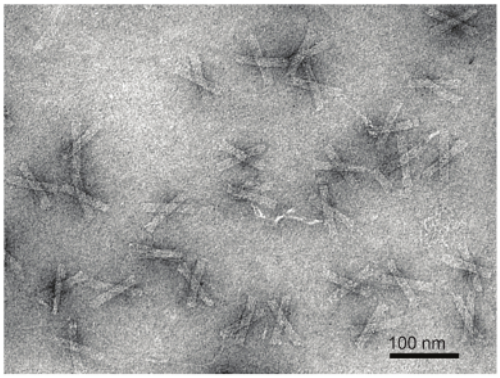

Figure 4: Representative TEM image of the DNA origami templates. The origami structure consists of two 14-helix bundles (80 nm x 16 nm x 8 nm) linked together by the scaffold strand. Please click here to view a larger version of this figure.

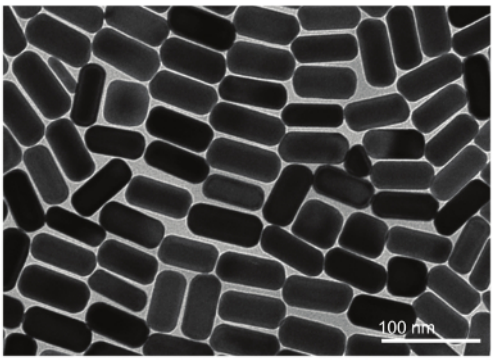

Figure 5: Representative TEM image of the AuNRs. The average dimensions of synthesized AuNRs are 70 x 30 nm. Please click here to view a larger version of this figure.

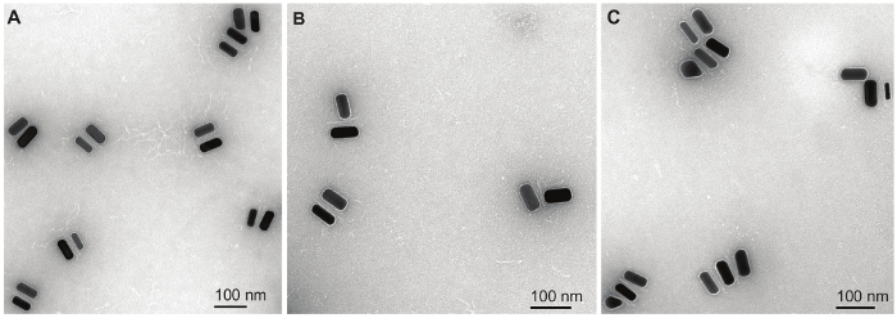

Figure 6: TEM images of origami-AuNR assemblies. (**A**) AuNR dimers on origami after annealing (band 1 in **Figure 3**). (**B**) AuNR dimers on origami without annealing (band 2 in **Figure 3**). (**C**) Origami-AuNR aggregates (band 3 in **Figure 3**). Please click here to view a larger version of this figure.

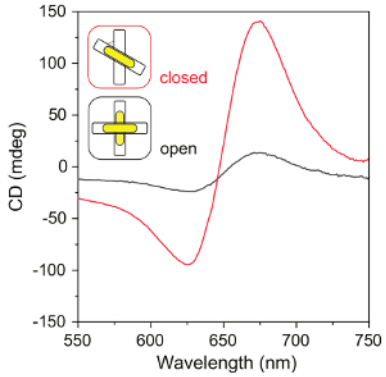

Figure 7: CD spectra of the origami-AuNR assemblies. The CD spectra of the closed structures (the origami templates fixed by lock strands into a right-handed configuration, with 50° between two origami bundles) and the open structure (the origami templates without lock strands). Please click here to view a larger version of this figure.

Discussion

The protocol introduces the whole workflow of design, assembly, purification, and characterization of DNA origami-based chiral assemblies of AuNRs. The DNA origami templates used in the protocol are particularly suitable for the fabrication of stimuli-responsive assemblies. Various types of responses and functionalizes can be incorporated into the lock strands that define the chiral state of the origami template (**Figure 1B**)[24,25,26,31]. For static assemblies, simpler block-shaped templates are often sufficient[14,45,46,47].

The DNA origami-based approach to the fabrication of plasmonic nanostructure inherits limitations of the DNA origami technique[48]. The size of the origami templates is typically limited by the size of the scaffold strand. The stability of DNA structures is reduced under law-salt conditions. The cost of synthetic stable strands remains rather high. However, recent developments in the field of structural DNA nanotechnology are expected to overcome these limitations[49,50,51,52,53,54,55].

Compared to other molecular-based approaches for generating chiral assemblies of AuNRs[34,35,36,37], DNA origami provides a high level of spatial precision and programmability.

For achieving reliable and reproducible optical responses of chiral assemblies, we strongly recommend adapting the protocols for AuNR synthesis[40], since the quality and optical properties of commercial products may vary between batches. Additional annealing (step 6.2) is often crucial for ensuring the correct attachment of AuNRs to DNA origami templates (**Figure 6**).

Finally, the protocol described here is not limited to chiral assemblies. DNA origami provides a very flexible platform for the fabrication of complex plasmonic nanostructures[9,10].

Disclosures

The authors have nothing to disclose.

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